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REVIEW

Use-dependent control of presynaptic calcium signalling at central synapses

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Abstract

Voltage-gated Ca^{2+} channels activated by action potentials evoke Ca^{2+} entry into presynaptic terminals thus briefly distorting the resting Ca^{2+} concentration. When this happens, a number of processes are initiated to re-establish the Ca^{2+} equilibrium. During the post-spike period, the increased Ca^{2+} concentration could enhance the presynaptic Ca^{2+} signalling. Some of the mechanisms contributing to presynaptic Ca^{2+} dynamics involve endogenous Ca^{2+} buffers, Ca^{2+} stores, mitochondria, the sodium–calcium exchanger, extraterminal Ca^{2+} depletion and presynaptic receptors. Additionally, subthreshold presynaptic depolarization has been proposed to have an effect on release of neurotransmitters through a mechanism involving changes in resting Ca^{2+}. Direct evidence for the role of any of these participants in shaping the presynaptic Ca^{2+} dynamics comes from direct recordings of giant presynaptic terminals and from fluorescent Ca^{2+} imaging of axonal boutons. Here, some of this evidence is presented and discussed.

Key words analogue coding; calcium buffers; calcium depletion; calcium dynamics; calcium stores; mitochondria; presynaptic receptors.

Introduction

Neurotransmitter release occurs via an exocytotic mechanism triggered by rises of the Ca^{2+} concentration inside the synaptic terminals (Sudhof, 2004). When neurons send chemical information to other neurons they do it through a fast chain of events (several milliseconds) that generally starts by the generation of currents at dendritic inputs (Fatt, 1957; Williams & Stuart, 2003). During their trip throughout the neuron these currents are integrated owing to the biophysical properties of the plasmatic membrane, and modulate excitability (Yuste & Tank, 1996; Gulledge et al. 2005). If the currents are excitatory and reach the initial segment of the axon, where there is a high density of Na^+ channels (Inda et al. 2006), a massive depolarization can occur, producing an action potential (Fuortes et al. 1957; Colbert & Johnston, 1996). If no failures occur during the propagation of the action potential, the axonal terminals will be briefly but intensely depolarized, boosting the intraterminal Ca^{2+} concentration (Koester & Sakmann, 2000), mainly through the activation of voltage-dependent Ca^{2+} channels, triggering the exocytotic machinery (Schneggenburger & Neher, 2005).

The increase of the intraterminal Ca^{2+} concentration disrupts the resting condition and triggers a number of mechanisms to re-establish the Ca^{2+} equilibrium: (1) endogenous Ca^{2+} buffers will trap some of the excess of Ca^{2+}, (2) Ca^{2+} pumps from three different sources (plasmatic membrane, endoplasmic reticulum and mitochondria) will use the energy of ATP to extrude Ca^{2+} from the cytoplasm, and (3) the Na^+/Ca^{2+} exchanger will generally use the electrochemical gradient of Na^+ ions to pump Ca^{2+} out of the presynaptic site. During these processes, the residual increase in Ca^{2+} concentration is likely to facilitate neurotransmitter release (Kamiya & Zucker, 1994).

Most plastic phenomena in synaptic transmission are related to the dynamics of presynaptic Ca^{2+} (if not directly explained by them). Although presynaptic Ca^{2+} dynamics has been intensely studied for many years, recent breakthroughs have come from electrophysiological recordings from single giant terminals (Geiger

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& Jonas, 2000) and from Ca^{2+} imaging in individual presynaptic boutons (Koester & Sakmann, 2000). Here, some well-documented mechanisms affecting presynaptic Ca^{2+} signalling, together with other contributing factors, are discussed.

Residual Ca^{2+} hypothesis and endogenous Ca^{2+} buffers

Paired pulse facilitation (PPF) is a short-term plasticity phenomenon occurring when the postsynaptic response to an action potential (AP) is larger than the response to a previous AP, the two stimuli being separated by less than a few hundred milliseconds. Katz & Miledi (1968) proposed that facilitation was caused by the slow decay of the Ca^{2+} transient following an AP so that if a subsequent AP occurs shortly thereafter, the residual Ca^{2+} adds to the new Ca^{2+} elevation, increasing the probability of release (Fig. 1A). This model was called the '(residual) Ca^{2+} hypothesis' and was proposed for the neuromuscular junction.

Experimental evidence has been reported extending this hypothesis to central synapses (Regehr et al. 1994; for a review see Burnashev & Rozov, 2005). Direct measurements of Ca^{2+}-dependent fluorescent transients in individual axonal boutons show that intraterminal Ca^{2+} dynamics after an AP is altered for several hundred seconds (Koester & Sakmann, 2000). The kinetics of free Ca^{2+} transients (Ca^{2+} available to trigger release) has been assessed by two-photon imaging in single hippocampal mossy fibre terminals, suggesting that high-frequency trains of AP elevate residual Ca^{2+} to about 0.2–1.5 μM during a train of five APs (Fig. 3; Scott & Rusakov, 2006). Normally, such Ca^{2+} rises do not to produce important (if any) release at central synapses but would be enough to induce facilitation when they are added to the local peak Ca^{2+} concentration (Bollmann et al. 2000; Schneggenburger & Neher, 2000).

Endogenous Ca^{2+} buffers are widely expressed in many neurons throughout the nervous system. These buffers are proteins such as parvalbumin, calretinin and calbindin with Ca^{2+} binding domains with different affinity for Ca^{2+}. Their presence and concentration in synaptic terminals can explain certain properties of neurotransmission, especially PPF. One of the proposed mechanisms responsible for the slow residual Ca^{2+} decay after an AP is the local saturation of the fast endogenous Ca^{2+} buffers in the terminal during a train of APs (Klingauf & Neher, 1997). Recently, this mechanism

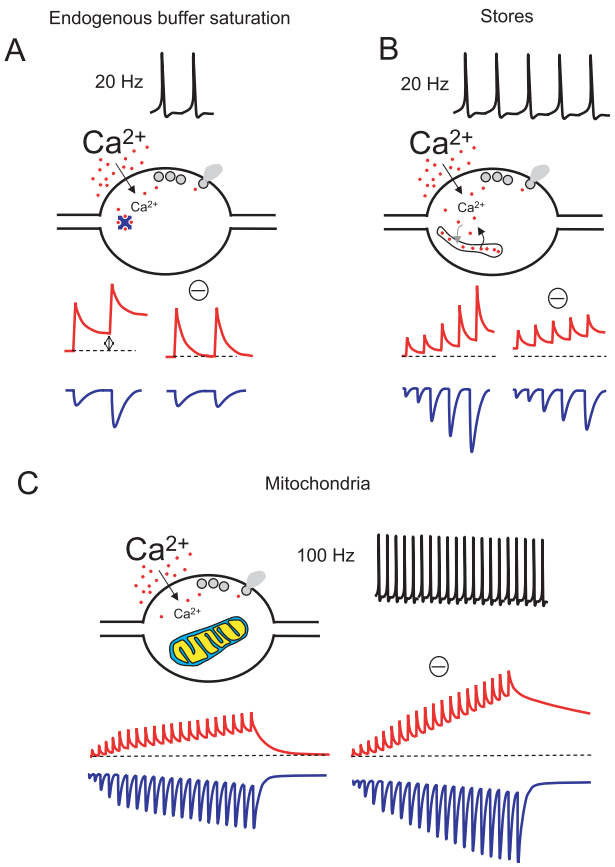

Fig. 1 Mechanisms of control of presynaptic Ca^{2+} dynamics (I). For all the panels in this figure and Fig. 2 a general idealistic illustration of each of the mechanisms described in the text is shown. The stimulating protocols at which these mechanisms occur are depicted on top of each panel. Red traces represent free Ca^{2+} concentration. Blue traces represent postsynaptic responses. The hypothetical blockade of each mechanism is indicated with a minus symbol ('–'). All panels represent general concepts and are not intended to show quantitative data. For an accurate idea of a realistic view of free Ca^{2+} dynamics and postsynaptic responses see Fig. 3. Only non-depressing synapses with a low release probability are considered. (A) The role of endogenous buffers in paired pulse facilitation (PPF) at a synaptic terminal. Two APs at 20 Hz produce two consecutive increases in Ca^{2+} concentration inside the terminal. The endogenous presynaptic Ca^{2+} buffers rapidly trap Ca^{2+} ions and can be partially saturated after the first AP at relatively high frequencies. Therefore, the residual Ca^{2+} when the second AP occurs is higher (left red trace), and this enhances transmitter release (left blue trace). The effect on free Ca^{2+} and EPSCs of a hypothetic blockade of buffer saturation is also illustrated. (B) Upon repetitive stimulation at 20 Hz, Ca^{2+} release from presynaptic Ca^{2+} stores can occur, contributing to the global Ca^{2+} signal. Through this mechanism neurotransmitter release is facilitated. The blockade of Ca^{2+} stores should reduce facilitation (only partially due to the presence of mechanism A) and increase resting Ca^{2+}. (C) Intense (100 Hz) stimulation recruits presynaptic mitochondria and contributes to buffer the excess of Ca^{2+}. In the absence of mitochondria the presynaptic Ca^{2+} concentration would reach higher levels during such a repetitive high-frequency stimulation, increasing the release rate.

has been shown to act at cortical calbindin-containing terminals (Blatow et al. 2003) as well as at giant calyx of Held synapses (Felmy et al. 2003).

Exogenous buffers have been widely used as a tool to investigate PPF. EGTA is a Ca^{2+} buffer which is too slow to compete with the fast Ca^{2+} sensor of the release machinery but fast enough to buffer residual Ca^{2+} during the interval between a pair of APs. Low concentrations of EGTA can abolish PPF (Blatow et al. 2003). Interestingly, terminals that contain parvalbumin, a slow endogenous buffer, do not show the strong facilitation occurring in parvalbumin knock-out mice (Caillard et al. 2000). The effect of slow buffers in PPF suggests that one of the requisites for a terminal to be facilitating is a long distance between the AP-dependent Ca^{2+} source and the Ca^{2+} sensor of the release machinery.

Unlike facilitation, short-term depression is generally explained by depletion of the ready releasable pool of vesicles in synaptic terminals. Therefore, the mechanism for depression does not seem to involve presynaptic Ca^{2+} dynamics. Consequently, for a terminal to be facilitating (in the context of the residual Ca^{2+} hypothesis) its release probability *p* should normally be low. At many synapses therefore repetitive activation results in an interplay between facilitation and depression of transmission.

Ca^{2+} stores

Ca^{2+}-induced Ca^{2+} release (CICR) mediated by ryanodine receptors (RyRs) from the sarcoplasmic reticulum has been well described in cardiac, skeletal and smooth muscles. In the brain, RyRs are localized in the endoplasmic reticulum (ER) at postsynaptic sites and glia (for a review see Verkhratsky, 2005). There is no general consensus on whether RyRs are located in presynaptic terminals in the brain, nor is it clear whether RyRs participate in the dynamics of intraterminal Ca^{2+}.

Although not universally, electron microscopy provides evidence for the presence of ER in presynaptic terminals (McGraw et al. 1980; Hartter et al. 1987; Lysakowski et al. 1999).

Carter et al. (2002) loaded bunches of hippocampal axons with membrane-permeable Ca^{2+}-sensitive dyes and directly measured (global) Ca^{2+}-dependent fluorescence transients (CaFT) induced by pairs of pulses. They reported no effect of thapsigargin and ryanodine (both drugs altering the Ca^{2+} store dynamics) in four different types of excitatory synaptic terminals from the hippocampus and cerebellum, including the mossy fibre large terminals in contact with CA3 pyramidal cells in the hippocampus.

However, Ca^{2+} imaging of individual terminals (rather than wide-field imaging of the bulk of fibres) in conditions of repetitive stimuli has revealed that these drugs could reduce the AP-evoked Ca^{2+} signal (Fig. 1B; Llano et al. 2000; Emptage et al. 2001; Liang et al. 2002; Scott & Rusakov, 2006). In addition, Ca^{2+} stores regulate the resting levels of Ca^{2+} at presynaptic terminals, thus playing a dual role, one more dependent and one less dependent on cell activity (Scott & Rusakov, 2006).

The evidence proposing a role for presynaptic CICR from stores in Ca^{2+} dynamics and therefore synaptic transmission is based on the effects of bath-applied ryanodine and thapsigargin (for a review see Bouchard et al. 2003). Because Ca^{2+} stores are present in dendrites and soma, the disruption of the Ca^{2+} dynamics in these compartments could affect the Ca^{2+} homeostasis in synaptic terminals simply because of diffusion of Ca^{2+} from one compartment to another. Recording at synaptic terminals located 1 mm or more far from the soma makes this problem less likely (Scott & Rusakov, 2006). However, such diffusional artefacts cannot be completely ruled out. A clear-cut demonstration of the functional role of presynaptic Ca^{2+} stores would require Ca^{2+} imaging inside the presynaptic stores.

Mitochondria

Blaustein et al. (1978) showed that a fraction of the Ca^{45} uptake in patched synaptosomes was associated with the intraterminal mitochondria. Whether mitochondria are important for transmitter release in the central nervous system has been a matter of intense studies during the last two decades. In a detailed electron microscopy study Shepherd & Harris (1998) showed that mitochondria are present only in < 50% of typical central presynaptic boutons from hippocampal CA1 axons, and a similar conclusion has been found by imaging mitochondria with fluorescent dyes captured by the organelle, in combination with imaging of FM dye destaining as an assay of neurotransmitter release (Waters & Smith, 2003). This evidence seems to raise the question if mitochondria are essential participants in the presynaptic function.

Direct imaging of mitochondrial and cytoplasmic Ca^{2+} in whole cell recordings of central giant terminals (calyx of Held) and simultaneous postsynaptic recordings have elegantly demonstrated a role of mitochondrial

Ca^{2+} sequestration in synaptic transmission in the brain (Billups & Forsythe, 2002; see also Fig. 1C). This phenomenon has also been reported in other systems (Tang & Zucker, 1997; David et al. 1998; David & Barrett, 2000), although not universally (Kobayashi & Tachibana, 1995; Zenisek & Matthews, 2000). Mitochondrial interaction with presynaptic Ca^{2+} dynamics seems to affect synaptic plasticity (Tang & Zucker, 1997; Levy et al. 2003), for instance, by maintaining transmission accelerating recovery from synaptic depression after periods of moderate activity (Billups & Forsythe, 2002).

Apart from their role in Ca^{2+} clearance during repetitive activity, mitochondria have also been proposed to act as Ca^{2+} reservoirs that are mobilized by the Na^+ influx during high-frequency and long-lasting stimulation in excitable cells. The increase of intracellular Na^+ concentration activates the mitochondrial Na^+/Ca^{2+} exchanger and induces Ca^{2+} efflux from the organelle (Rizzuto, 2003; Yang et al. 2003).

By directly patching mitochondria inside synaptic terminals of the giant squid axon, Jonas et al. (1999) detected large conductances (several nS) occurring with an occasional frequency that increased up to 60-fold during trains of AP several seconds long at high frequency, and continued to increase after the stimulation had stopped, to recover after tens of seconds. Interestingly, when mitochondria become overloaded with Ca^{2+}, they can undergo the so-called mitochondrial permeability transition. This includes formation of a non-selective pore that allows solutes of 1500 Da or smaller to pass through the inner mitochondrial membrane with a resultant rupture of the outer mitochondrial membrane caused by osmotic swelling. If occurring in axonal mitochondria, such mechanisms would affect presynaptic Ca^{2+} dynamics. Interestingly, synaptic mitochondria seem to be more susceptible than somatic or dendritic mitochondria of suffering mitochondrial permeability transition (Brown et al. 2006).

Na^+/Ca^{2+} exchanger

There is evidence supporting a functional role for the presynaptic Na^+/Ca^{2+} exchanger (NCX) in cultured hippocampal neurons (Reuter & Porzig, 1995; Doi et al. 2002), in cerebellar granule cells (Doi et al. 2002; Regehr, 1997), in neurohypophysis (Lee et al. 2002) and in the calyx of Held (Kim et al. 2005).

The NCX has a high capacity for Ca^{2+} transport, but a low affinity for Ca^{2+} (Allen et al. 1989). It exchanges three Na^+ ions for one Ca^{2+} ion and, in the case of the $Na^+/Ca^{2+}K^+$ exchanger (NCKX), four Na^+ ions for one Ca^{2+} and one K^+ ion. This electrogenic mechanism, depending on the membrane potential and the intracellular and extracellular concentrations of Na^+ and Ca^{2+} (and K^+), can either remove Ca^{2+} from the cell or transport Ca^{2+} from the extracellular space into the cytoplasm. Actually, there is evidence showing that presynaptic residual Ca^{2+} after a period of high-frequency activity is caused by Ca^{2+} influx through a reverse mode Na^+/Ca^{2+} exchange (Zhong et al. 2001).

Study of the role of NCX in central synapses has been difficult owing to the lack of specific pharmacology. Using Na^+- and Ca^{2+}-sensitive fluorescent indicators it has been possible to record spike-evoked Na^+ and Ca^{2+} presynaptic transients in granule cell parallel fibres in brain slices from rat cerebellum. A model of the kinetics of the NCX and the Ca-ATPase at presynaptic terminals fitted well with the recorded Na^+- and Ca^{2+}-dependent fluorescence transients. Immediately following stimulation, the NCX removed Ca^{2+} from the terminal more rapidly than does the Ca-ATPase. However, eventually, the large Na^+ influx drives the exchanger into steady state, being the Ca-ATPase the only one to extrude Ca^{2+}. This disrupts the equilibrium of the NCX, which acts opposite to the Ca-ATPase, and thus Ca^{2+} and Na^+ slowly return to resting levels (Regehr, 1997).

At the Calix of Held, Kim et al. (2005) compared the presynaptic Ca^{2+} removal after a 50-ms voltage depolarization in the presence of normal extracellular Na^+ concentration or the same concentration of Li^+. They also studied the effect of substituting internal K^+ by tetraethylammonium (TEA). Their results suggest that, in response to small Ca^{2+} transients (2 μM), Ca^{2+} loads are cleared from the calyx of Held primarily by NCKX (42%) and NCX (26%). Additionally, they conclude that in these conditions plasmatic membrane Ca^{2+} ATPase participates with 23% of Ca^{2+} clearance, and that mitochondria participate when the Ca^{2+} load is larger or prolonged.

New pharmacological selective tools are needed to characterize further the role of presynaptic NCX with different patterns of stimulation.

Ca^{2+} depletion in the synaptic cleft

A potentially important mechanism of regulating presynaptic (and postsynaptic) Ca^{2+} dynamics is depletion of the extracellular concentration of Ca^{2+} in the synaptic cleft due to Ca^{2+} flux from the synaptic cleft into the

pre- and postsynaptic sites (Fig. 2A). This would reduce the driving force for Ca^{2+} and, during high-frequency activity, would diminish subsequent Ca^{2+} influx.

The concentration of Ca^{2+} in the extracellular volume of the brain decreases during repetitive activity in both physiological and pathophysiological conditions (Nicholson et al. 1978; Krnjevic et al. 1980; Heinemann et al. 1986).

Changes of Ca^{2+} concentration in the synaptic cleft have been theoretically predicted (Smith, 1992; Vassilev et al. 1997; Egelman & Montague, 1998; Rusakov et al. 1998). Indeed, several lines of experimental evidence propose that such reductions in presynaptic Ca^{2+} influx may occur due to activity-dependent depletion of Ca^{2+} in the synaptic cleft of calyceal synapses (Borst & Sakmann, 1999; Stanley, 2000; Rabl & Thoreson, 2002). Additionally, in more common types of central synapses depletion of extracellular Ca^{2+} was optically measured in response to brief trains of APs. This phenomenon seems to be due to postsynaptic NMDA receptor activation and modulates presynaptic release (Rusakov & Fine, 2003).

A role for the glial sheath covering synapses has been proposed to enhance Ca^{2+} depletion in the synaptic cleft in a computational model (Rusakov, 2001). Finally, it has recently been shown that depletion of extracellular Ca^{2+} can regulate neuronal Ca^{2+}/CaM-dependent protein kinase via a depletion of intracellular stores (Cohen & Fields, 2006). Whether enzyme activities in synaptic terminals can be modulated during brain activity by extracellular Ca^{2+} depletion requires further investigation.

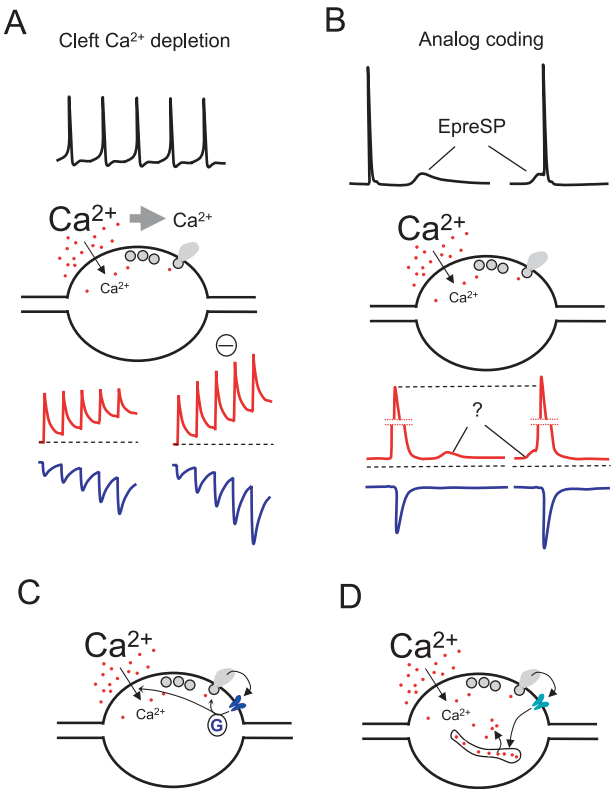

Fig. 2 Mechanisms of control of presynaptic Ca^{2+} dynamics (II). See also legend to Fig. 1. (A) High-frequency stimulation transiently decreases the extracellular concentration of Ca^{2+} in the synaptic cleft due to Ca^{2+} ions passing into the cells during the electrical activity. The reduction of the electrochemical gradient for Ca^{2+} produces a decrease of presynaptic Ca^{2+} entry during subsequent AP and a reduction of neurotransmitter release. A hypothetical blockade of Ca^{2+} depletion restablishes the amplitude of consecutive Ca^{2+} transients and enhances facilitation. (B) Subthreshold voltage changes in the dendrites or soma can passively travel relatively long distances along the axons and regulate neurotransmitter release when they immediately precede an AP. A suggested mechanism for this phenomenon would be that small changes in resting Ca^{2+} produced by such subthreshold voltage signals would add to the Ca^{2+} transient elicited by an AP, resulting in an enhancement of transmitter release. (C) Presynaptic metabotropic receptors can modulate Ca^{2+} entry and or neurotransmitter release via G-proteins. (D) Ionotropic receptors can regulate neurotransmitter release interacting with intracellular stores.

Presynaptic receptors

Presynaptic receptors (metabotropic as well as ionotropic) affecting Ca^{2+} dynamics at synaptic terminals have been investigated by using intracellular Ca^{2+} chelants and, more recently, by directly imaging presynaptic boutons. Presynaptic group II and III metabotropic glutamate receptors modulate excitatory transmission by decreasing AP-dependent presynaptic Ca^{2+} transients at area CA1 synapses in the hippocampus (Faas et al. 2002). They could act on both cytoplasmic and membrane-bound effectors by differential activation of either diffusible $G\alpha$ proteins or the membrane-associated $G\beta\gamma$ subunits, respectively, or both (Fig. 2C). Potentially, these actions of mGluRs could affect both the release cascade directly (mainly through $G\alpha$ signalling) or by down-modulating Ca^{2+} channels (mainly through $G\beta\gamma$ signalling).

Presynaptic ionotropic receptors (Fig. 2D), like kainate receptors (KARs), also affect presynaptic Ca^{2+} signalling (Kullmann, 2001). Ca^{2+} imaging of the bulk of mossy fibres in hippocampal area CA3 suggests that activation of KARs reduces presynaptic Ca^{2+} transients (Kamiya et al. 2002). Consistent with this finding, electrophysiological recordings suggest a KAR-dependent triggering of Ca^{2+} release from internal stores at mossy fibre

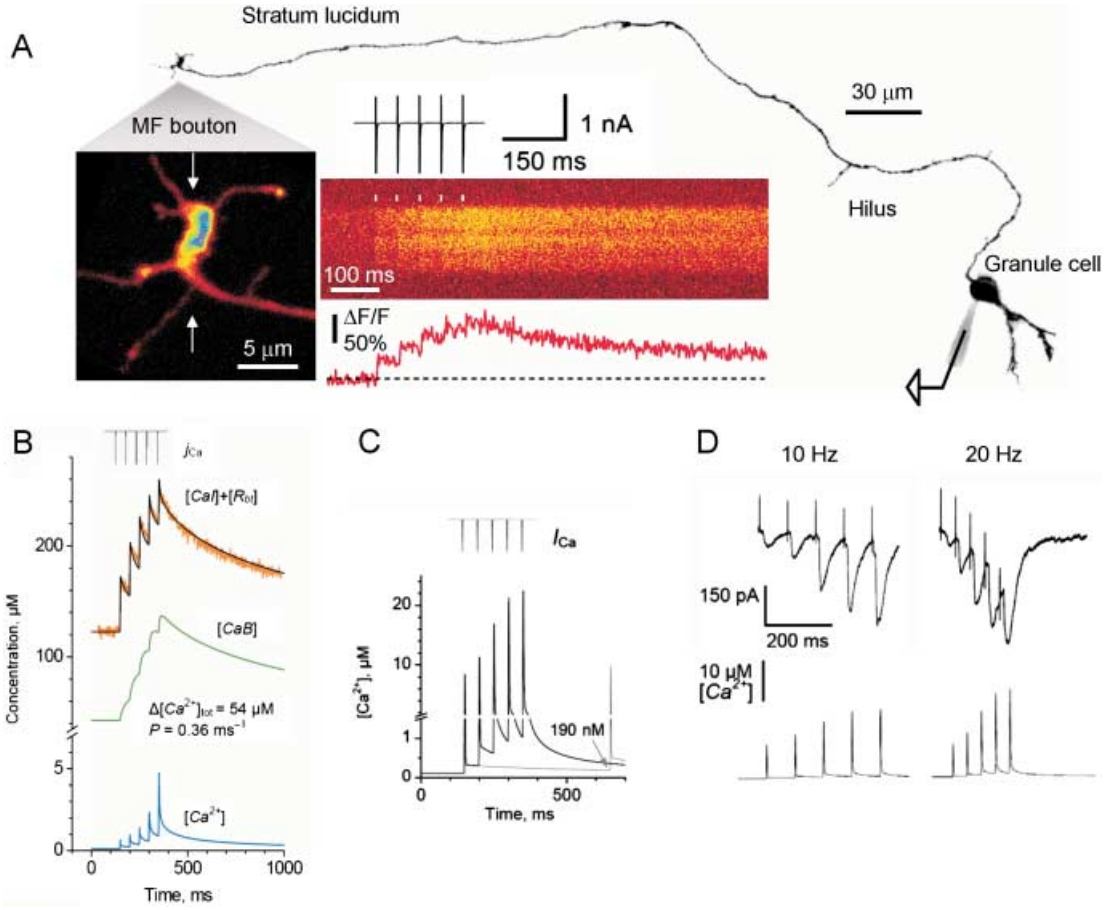

Fig. 3 Ca^{2+} dynamics in mossy fibre giant terminals at the stratum lucidum. (A) Giant terminal (with visible filopodia) emerging from the mossy fibre of a granule cell loaded with alexa-594 and fluo-4 through a patch pipette. The morphology of the cell and the main axon is shown (collateral branches were out of focus). The cell body was briefly depolarized evoking action currents that propagated along the axon producing Ca^{2+}-dependent fluorescence transients at the giant terminals, almost 1 mm distant. These transients were recorded by line-scanning two-photon microscopy. The average of ten fluorescent transients is shown. (B) Average response for 18 giant boutons (orange trace) in similar conditions to A. Several parameters involved in Ca^{2+} dynamics could be assumed (AP-dependent Ca^{2+} influx, calbindin concentration, dye concentration, and K_d of the dye; Scott & Rusakov, 2006). An accurate fitting of the experimental data was obtained, also rendering the kinetics of the endogenous buffer bound to Ca^{2+} (green trace) and the free Ca^{2+} concentration changes during trains of AP (blue trace). In order to constrain the unknown parameters (total Ca^{2+} entry and removal rate, $\Delta[Ca]_{tot}$ and P, respectively), the experiments were repeated with fluo-4 (50 μM) and fluo-5 (200 μM). Similar values were obtained in the three conditions for these unknowns (not shown). (C) The model allowed us to extrapolate the dynamics of free Ca^{2+} concentration in the absence of exogenous buffers. It was also possible to simulate the free Ca^{2+} dynamics during different frequencies of AP. (D) Typical EPSCs recorded in CA3 pyramidal cells at 20 Hz and 50 Hz. Below, the simulations of free Ca^{2+} at the corresponding frequencies (modified from Scott & Rusakov, 2006).

synapses (Lauri et al. 2003). Besides the extensively studied KARs, other types of ionotropic receptors have also been implicated in presynaptic modulation of transmission at central synapses. In the cerebellum, presynaptic NMDA receptors enhance GABA release at inhibitory synapses onto Purkinje cells (Duguid & Smart, 2004) and also contribute to long-term depression at parallel fibre–Purkinje cell synapses (Casado et al. 2002). A role for presynaptic NMDA receptors in synaptic depression has also been described in neocortical pyramidal cells (Sjostrom et al. 2003). Regulation of neurotransmitter release by presynaptic GABAA receptors, a well-established mechanism of inhibition of presynaptic activity in the spinal cord, has been found to occur in the calyx of Held (Turecek & Trussell, 2001) and in developing cerebellar interneurons (Pouzat & Marty, 1999). In individual axonal boutons from hippocampal mossy fibres, GABAA receptors reduce presynaptic AP-induced Ca^{2+} entry and elevate resting Ca^{2+} (Ruiz et al. 2003). Depending on the intracellular concentration of Cl^-, the activation of these receptors could either depolarize or hyperpolarize the

terminals allowing, in principle, a bi-directional control of the release probability.

Analogue coding

Neurotransmitter release at synaptic terminals located at relatively short distances can occur or be modulated by subthreshold changes of the somatic or dendritic membrane potential. Such a graded transmission (in contrast to transmission due to APs) occurs in small invertebrates (Juusola et al. 1996) and it is possible in part because of the specific expression of presynaptic L-type Ca^{2+} channels, which activate relatively rapidly and do not (or almost do not) inactivate.

Recently, a graded (also termed analogue) modulation of synaptic transmission has been described for neurons in the mammalian brain (Alle & Geiger, 2006; Shu et al. 2006). Subthreshold voltage changes caused by synaptic activity in dendrites can be passively transmitted along the axon and reach far distances due to the long length constant of the axon (~430 μm). These voltage signals, when overlapping with an AP, enhance transmitter release (Fig. 2B). Because intracellular EGTA decreases this facilitation, it has been suggested that analogue coding could be related to presynaptic Ca^{2+} changes. Nevertheless, the reduction of facilitation in the presence of intracellular EGTA could also be explained by a combined voltage and Ca^{2+} dependence of the release machinery. At mossy fibre terminals in area CA3, Ca^{2+} channels do not seem to activate at voltages more negative than –60 mV (Bischofberger et al. 2002). This appears to be inconsistent with an effect of subthreshold dendritic activity on Ca^{2+} channels from axonal terminals located 500–1000 μm from the soma, assuming a V_m of approximately –80 mV at granule cell soma and terminals and a length constant of 430 μm for the mossy fibre (Alle & Geiger, 2006). At the calyx of Held, subthreshold depolarization of the terminals produces Ca^{2+} entry through P/Q-type Ca^{2+} channels, thus enhancing neurotransmitter release (Awatramani et al. 2005). Direct evidence for subthreshold resting Ca^{2+} changes in axonal varicosities has been reported for the hippocampal mossy fibre up to 200 μm from the soma. However, these changes in resting Ca^{2+} were detected when the soma underwent long (tens of seconds) periods of subthreshold depolarization (Ruiz et al. 2003). Additionally, somatic effects on AP-dependent Ca^{2+} signal have been reported for this axon, occurring with a length constant of ~175 μm (Scott & Rusakov, 2006). To determine whether Ca^{2+} signalling could underlie the mechanism of analogue electronic control in these conditions, it would be advantageous to monitor the dendrosomatic subthreshold effects on presynaptic Ca^{2+} dynamics – mediated by Ca^{2+} channels, Ca^{2+} stores or other mechanisms – directly in individual remote terminals.

Concluding remarks

A number of mechanisms, which often interact with one another, take part in controlling presynaptic Ca^{2+} dynamics in different conditions. An ideal research objective would be to build a mechanistic model describing the integrated effect of these mechanisms in the intraterminal Ca^{2+} concentration for specific synapses. Novel imaging techniques will surely provide interesting data on local presynaptic Ca^{2+}, and, ultimately, on the integration of the kinetics of Ca^{2+} microdomains with exocytotic events. In the next few years our knowledge of the dynamics of presynaptic Ca^{2+} will reach the submicrometre resolution for several types of synapses. However, many questions will remain. How could the signature of presynaptic Ca^{2+} dynamics of certain synapses affect neural circuits? Can these circuits alter their pattern of activity by changes in presynaptic Ca^{2+} dynamics of some (how many?) of their individual synapses? Would these network changes occur during development and during leaning?

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